United States Environmental Protection Agency | Region 9 & Office of Water | EPA-xxx-x-xx-xxx November 2018

EPA Response to the

External Peer Review of U.S. EPA's

"*Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California*"

(xx November 2018)

U.S. Environmental Protection Agency Region 9
Water Division
San Francisco, CA

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, D.C.

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I. INTRODUCTION

The U.S. EPA Office of Water is charged with protecting ecological integrity and human health from adverse anthropogenic, water-mediated effects, under the purview of the Clean Water Act (CWA). In concurrence with this mission, in 2016 EPA finalized the recommended freshwater aquatic life chronic tissue based selenium criterion for egg-ovary, whole-body and/or muscle concentrations in fish. Selenium toxicity studies have been conducted on a wide diversity of organisms, including numerous species of fish and birds, indicating that exposure to elevated concentrations of selenium through the aquatic food chain can cause population level effects, such as reproductive impairments. In the 2017 document, "Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California," EPA presented a draft water quality criterion for the state of California, using the previously peer reviewed final national selenium ambient water quality criterion to ensure the protection of aquatic life species and providing support for the derivation of selenium criteria that would be protective of aquatic-dependent wildlife species. Specifically, EPA is: 1) proposing the previously peer reviewed and published 2016 final national selenium freshwater ambient water quality criterion to ensure the protection of aquatic life species; and 2) providing support for and summarizing the derivation of a selenium criterion that would be protective of aquatic-dependent wildlife.

EPA funded a contractor-led and independent external peer review of the derivation of the aquatic-dependent wildlife criterion and the translation of the aquatic life and aquatic-dependent wildlife criterion to a water column concentration using the mechanistic bioaccumulation model in EPA's Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for the state of California.

The contractor selected five senior scientists to serve as peer reviewers from December 2017 through February 2018, with expertise/experience in one or more of the following disciplines, especially with respect to ecological impacts of selenium on aquatic life and aquatic-dependent wildlife: (1) toxicity of selenium in aquatic life and/or aquatic-dependent wildlife, (2) aquatic ecotoxicology; (3) statistical analyses and data interpretation for the determination of data acceptability; and (4) environmental occurrence and fate of selenium in the environment.

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II. CHARGE PROVIDED TO EXTERNAL PEER REVIEWERS

Selenium toxicity studies have been conducted on a wide diversity of organisms, including numerous species of fish and birds, indicating that exposure to elevated concentrations of selenium through the aquatic food chain can cause population-level effects, such as reproductive impairments. In this draft, EPA is proposing water quality criteria for the state of California using the previously peer reviewed final national selenium ambient water quality criteria to ensure the protection of aquatic life species and providing support for the derivation of selenium criteria that would be protective of aquatic-dependent wildlife species. In this peer review, EPA is seeking to obtain a focused, objective evaluation of the criteria derived to protect aquatic-dependent wildlife and the translation of the aquatic life criteria to water column selenium concentrations in California intended to protect both aquatic life and aquatic-dependent wildlife. (The 2016 fish-tissue based Final Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater is *NOT* the subject of this peer review.)

1. Please comment on the overall clarity of the document and construction as it relates to assessing the risk to aquatic-dependent wildlife in California.

2. Please comment on the technical approach used to derive the draft criteria for aquatic-dependent wildlife presented in EPA's Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California. Is the technical approach used to derive the aquatic-dependent wildlife criteria logical? Does the science support the conclusions? Is it consistent with the protection of aquatic-dependent wildlife?

3. Please comment on the toxicity data for aquatic-dependent wildlife used to derive the aquatic-dependent wildlife criteria presented in the draft document. Were the data adequately used and sufficiently comprehensive to represent risks to aquatic-dependent wildlife? Were the data selected and/or excluded from the criteria derivation appropriately utilized? Are there relevant data that you are aware of that should be included? If so, please provide for consideration.

4. Please comment on the approach used to derive the EC_{10} described in this draft document of 13.1 mg/kg dw based on mallard toxicity data. Is the EC_{10} of 13.1 mg/kg dw protective of aquatic-dependent wildlife?

5. Please comment on the use of the USGS Ecosystem-Scale Selenium Model to derive the water column criterion elements for aquatic life and aquatic-dependent wildlife considering the fate and transport of selenium. In particular, please comment on:

> 5a. Any uncertainty surrounding the use of site-specific enrichment factors (EFs) (also commonly known as Kds) for California lentic and lotic water bodies.
>
> 5b. Any uncertainty surrounding the use of species specific trophic transfer factors (TTFs) in the food chain of aquatic life and aquatic dependent wildlife found in California.

6. Please comment on the science provided in EPA's Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California regarding the utility of the derived criteria for aquatic life and aquatic-dependent wildlife found in California with respect to the protection of listed threatened and endangered species from potential effects of selenium exposure.

III. PEER REVIEWER COMMENTS AND EPA RESPONSES BY CHARGE QUESTION

General Impressions		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
1	Overall, the Criteria document is well organized, well written, and comprehensive in its review of selenium aquatic toxicology in general and with our current understanding of relevant ecotoxicology, fish toxicology, duck and aquatic bird toxicology found in controlled studies and studies of Se impacted aquatic ecosystems. The Criteria Document explores the relevant peer-reviewed knowledge base for freshwater selenium effects in lentic and lotic systems, and the related literature of direct observation of impacted ecosystems and in controlled Se dose-response studies. The Criteria document has a strong problem formulation, review of the aquatic ecosystem and aquatic ecosystem dependent effects, and development of criterion leveraging the recently finalized USEPA selenium criteria. The Criteria document performs a sufficiently robust review of the 2016 fish-tissue based Final Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater (Part 3). The Criteria Document Part 4 then expands the discussion to the development of aquatic-dependent wildlife, primarily avian species. This included a reanalysis of older data and the presentation of more recent data, with a focus on reproductive studies; this is a solid effort in assembling the known dataset of published and agency reported work. In preparation for this review of Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California(22 December 2017), herein referred to Criteria Document, I read and annotated the Criteria document and reviewed major cited references. Also, I performed a Web of Science database search of the most recent scientific literature (January 2017-present) using the search terms selenium AND fish, OR duck OR aquatic bird. The results of this literature search suggest that the authors of the	Both the EPA's 2016 aquatic life criteria (U.S. EPA 2016) and DeForest et al. (2017) translation procedures are based on calculating TTFs across consumer and diet organisms within aquatic food webs and calculating EFs between particulate and water. In U.S. EPA (2016), species-specific fish and invertebrate TTFs were calculated as the median ratio of all available consumer-resource paired data applicable to that species. EFs were calculated at each site using site-specific data. Next, food webs were constructed at each site for every species of fish sampled at that site and these were parameterized with TTFs calculated using all available paired data. The lowest translated water concentration was selected for each site, and then a conservative centile (20th) was applied to the distribution of all lentic and lotic sites, respectively. In DeForest et al. (2017), paired data from adjacent trophic levels were analyzed using a quantile regression model. A 75^{th} centile was used to instill some conservatism into the model. For the fish to invertebrate regression, the model was

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	Criteria Document did a very good review of the available reports and peer-reviewed literature. Because of the well understood time limitations of putting a comprehensive and internally reviewed Criteria Document together, there is always the potential that the very latest works could be missed. My literature search suggests that no major data source impacting the input and conclusions of the Criteria Document was published in January 2017 to the present date of this review except one highly relevant September 2017 paper. This work is DeForest, et al. *Lentic, lotic, and sulfate-dependent waterborne selenium screening guidelines for freshwater systems* (Environmental Toxicology and Chemistry, Volume 36, Issue 9. September 2017. Pages 2503–2513). A complete reprint copy of is presented as an appendix to this review. Peer-reviewed analyses, observations and outcomes relevant to the Criteria Document and therefore the conclusions are raised recently raised in the DeForest et al. September 2017 paper *Lentic, lotic, and sulfate-dependent waterborne selenium screening guidelines for freshwater systems.* This work challenges the omittance of sulfate as a biogeochemical consideration in selenate containing freshwater effects modeling and criteria (guideline) development and proposes an alternative approach. Although the present task is not a review of 2016 fish-tissue based Final Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater, DeForest et al. (2017) also apply a multi-step partitioning approach to derive protective lentic and lotic water column concentrations of selenium with results about two-fold greater than the present work. The authors of the Criteria Document should address the observations and results of DeForest et al. (2017) directly in the final document, highlighting the strengths and weaknesses. The conflict of approaches and conclusions in the Criteria Document and the DeForest, et al. (2017) paper are not easily resolved in the scope of this	solved for the invertebrate concentration corresponding to a target fish concentration. The same modeling approach was also applied to paired invertebrates and particulates, and paired particulates and water. In short, a somewhat conservative quantile (75th) was applied to all trophic relationships, and to the water-particulate relationship analyzed in aggregate, to translate from a target fish concentration, to the corresponding invertebrate, particulate, and water concentration, in succession. These regressions were applied separately to each site. In summary, U.S. EPA (2016) calculated taxa specific TTFs and site specific EFs, then applied a conservative centile to the distribution of all sites to calculate a final translated water concentration. DeForest et al. (2017) applied a conservative quantile regression model to consumer-diet and particulate-water paired data combined in aggregate. The end result of these approaches were very similar translated water concentrations, when translating from a fish egg ovary tissue concentration of 15.1 mg/kg, with the EPA approach resulting in lentic and lotic water

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	present review. The relative absence or minimization of sulfate effects in much of the aquatic ecotoxicology research over the past 25 years – given their similar biogeochemistry and periodicity – has always been a significant data gap. Thus, the work of DeForest et al. (2017) is refreshing in that regard. The DeForest et al. (2017) 75th quantile of the multiple quantile regression model approach appears as a valid alternative but does not invalidate the well-developed USGS Ecosystem-Scale Selenium Model, as is sometimes the situation for competing models. A thorough comparative analysis beyond the scope of this present review may be required. The conclusions of the Criteria Document are sound if the authors can sufficiently present such a comparative analysis and argument, perhaps in brief.	concentrations of 1.5 µg/L and 3.1 µg/L, and the DeForest et al. (2017) approach resulting in lentic and lotic water concentrations of 1.7 µg/L and 2.8 µg/L. A second difference between Deforest et al. (2017) and U.S. EPA (2016) is that DeForest et al. also included a sulfate adjustment to the translated water concentrations. The sulfate correction does not apply to the comparison described above, however. Reasons for not including a sulfate adjustment were discussed in Section 6.2.2 in U.S. EPA (2016). One reason for not including a sulfate adjustment was the lack of laboratory data examining the effect of sulfate on selenium toxicity in periphyton and benthic diatoms, limiting a comprehensive evaluation of the effects of sulfate on bioconcentration and transfer through food webs. A second reason was that a paired sulfate-selenium water measurement would have been required at all sites used to perform the tissue to water translation, and that the reduction in sample size would reduce the confidence in the translated water values.
2	This was generally a well-written document describing the derivation of water quality criteria for selenium in inland surface waters, enclosed bays	Reviewer No. 2 made several comments all related to the California aquatic life and

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	and estuaries of California. The authors appeared to begin to use an Ecological Risk Assessment framework to discuss the derivation of the criteria. The Problem Formulation section was well developed and included appropriate background on occurrence of selenium in California. However, this section dealt specifically with inland waterways and did not appear to have coastal values discussed. Overviews of the importance of speciation and bioaccumulation were well documented. The sections concerning the mode of action was somewhat limited and did not contain updated information that may be important particularly for water bodies influenced by hypersaline conditions. Similarly, the conceptual model is sound for freshwater systems, but may not be appropriate for brackish water ecosystems. On the effects assessment section, the species of concern are all primarily freshwater species with *Oncorhynchus and Acipenser* potentially being estuarine or species. However, even in these species, tissue based criteria are derived from freshwater exposures. In contrast, use of the mallard duck as a sensitive model species for derivation is appropriate as this species does inhabit fresh and brackish water systems and is likely unaffected by salinity. Back calculation from tissue-based criteria to water column criteria appears to be sound, but needs additional discussion on the uncertainties associated with the calculation, particularly if an Ecological Risk Assessment paradigm is to be used. In addition, a weight of evidence section is likewise absent within Effects and Exposure Assessment sections, and an overall Risk Characterization component where uncertainties can be discussed was absent. Overall, the estimates for freshwater criteria appeared to be appropriate for freshwater, but were limited for saltwater/estuarine systems. It was also unclear how "Enclosed Bays" were defined. Similarly, estuarine systems were also uncharacterized.	aquatic-dependent wildlife selenium document not considering or including saltwater data in the derivation of the criteria. Only freshwater data were used in this document to derive the tissue-based criteria and translate the tissue-based criteria to water elements. EPA agrees with the reviewer that the original title suggests saltwater and estuaries are included and so the title has been revised accordingly. Unfortunately, there are not enough data to derive a separate saltwater tissue-based criterion for either aquatic life or aquatic-dependent wildlife. In Chapter 6 of Ecological Assessment of Selenium in the Aquatic Environment (Chapman et al. 2010), Section 6.5.4 examines Marine vs Freshwater Environments in which several studies are discussed that suggest marine animals are not as sensitive as freshwater animals. For additional details regarding on how the proposed criteria are applied to waters in California, please see Section III of the proposed rule. Regarding the comment on the effects assessment of freshwater and no saltwater exposure to *Oncorhynchus* and *Acipenser*, EPA is not aware of any acceptable maternal transfer studies with saltwater

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		exposures to these fish. Please see EPA's response to the comment under Charge Question 3, Reviewer No. 2 for additional information. Regarding the comment on a weight of evidence approach being absent from the Effects and Exposure Assessment sections, EPA did not include in the Draft California selenium TSD much of the toxicity information that is given in the U.S. EPA (2016) ALC document that provides evidence supporting the tissue criterion. The CA document focused on the reproductive toxicity data that were used to derive the criterion. Section 6 of the 2016 ALC discusses all the acceptable reproductive toxicity data not used to derive the criterion (i.e., not the four most sensitive), acceptable non-reproductive toxicity studies, a comparison between the reproductive and non-reproductive effect levels, juvenile salmonids and other topics. The following sentences have been added to Part 3.2 of the CA TSD that direct the reader to two sections in the 2016 ALC document for toxicity information that

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		supports the tissue criterion. "This section presents a summary of reproductive studies included in the selenium data set and how they were used to derive the tissue criterion elements for egg-ovary, whole body and muscle. For a detailed review of each reproductive study used to derive the criterion, see Section 3.1.1 - Acceptable Studies of Fish Reproductive Effects for the Four Most Sensitive Genera, in U.S. EPA (2016). Other reproductive and non-reproductive studies that support the derivation of the tissue criterion are provided in Section 6 - Effects Characterization, of U.S. EPA (2016)."
3	The draft criteria document meets or exceeds expectations for a criteria document. For the most part, it is very clearly written, consistent with currently-accepted best approaches, draws sound and defensible conclusions, and innovatively applies a tissue-to-water approach for an element available primarily through trophic exchange. Consequently, many of the specific comments provided below are primarily intended as refinements to consider in future documents. The historical outline of selenium criteria development is clear and establishes a logical foundation for the proposed tissue-based approach. The rationales are sound for using total selenium concentrations and emphasizing dosing studies that apply organic selenium. The major sources and geographical distribution of high selenium waterbodies are explained in	EPA thanks you for your review and comment. EPA acknowledges the comments and recommendations provided by the reviewer on providing "richer exploration of variation anticipated around estimates and predictions" in future criteria documents. EPA discussed uncertainty of the tissue criterion elements (egg ovary, muscle and whole body), conversion factors, TTFs, EFs and water values in Section 6.3 of the Aquatic Life AWQC for

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	good detail. Speciation discussion gives enough insight to justify separate consideration of lentic and lotic systems, and for the focus on food web exposure. The "*narrow margin between sufficiency [of Se in diet] and toxicity*"(page18) was explained, yet started this reviewer wondering why, given this narrow margin, more attention was not paid to exploring uncertainty in estimates used throughout later parts of the document. The difference between sufficient and toxic concentrations was "*approximately an order of magnitude*" (page 19). Were the uncertainties in estimates and predictions in this report more or less than an order of magnitude? As an instance in which presentation of such uncertainty information would have been useful (pages 34-35), a 5th percentile projection of 15.1 mg Se/kg egg-ovary is compared to a most sensitive species value of 15.6 mg Se/kg. The same comparison is then done for whole-body concentration (8.5 versus 9.2 mg Se/kg). Would confidence or credible intervals of these 5th percentile projections overlap with those of the most sensitive species? It would also be helpful to understand how the uncertainties associated with parameter estimations influence the range of predictions from the USGS Ecosystem-scale selenium model. The EPA should aspire to eventually provide a richer exploration of variation anticipated around estimates and predictions. Perhaps Monte Carlo resampling methods could be applied to this end. The Agency might also try to move away from heavy reliance on conventional Null Hypothesis Significance Tests (NHSTs) that are being questioned with increasing frequency by many scientists and statisticians alike (e.g., the American Statistical Association's position as stated in Wasswestein & Lazar, 2016, *The American Statistician*, 70, 129-133). This EPA draft document applies the species sensitivity distribution with a small number of effect metrics to derive "[c]*riteria … intended to be*	Selenium (U.S. EPA 2016). The uncertainty discussion in Section 6.3 applies to the aquatic life tissue criterion and translation to water values (CFs, TTFs and EFs) in the Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Freshwaters of California.

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	protective of a majority of aquatic organisms in the community (i.e., approximately the 95th percentile of tested aquatic organisms or aquatic-dependent wildlife representing the aquatic community) … the health of the aquatic ecosystem may be considered as an assessment endpoint indicated by survival, growth, and reproduction [of individual organisms]" (page 22). It states also that, "*The typical assessment endpoints for aquatic life and aquatic-dependent wildlife criteria are based on effects on growth, deformity rates, reproduction, or survival of the assessed taxa. These measures of effect on toxicological endpoints of consequence to populations …*" The EPA draft document uses the species sensitivity distribution context which is a widely accepted regulatory approach. Regardless of its pervasive application, it is scientifically unjustifiable to conclude from the results that only 5% of species in the entire community remain unprotected and that ecological effects such as those involving population demographics, community interactions, prey switching, and trophic cascades can remain unexamined. This is especially the case given the crucial role of trophic ecology in dictating selenium exposure. The wording in the draft document could be modified to acknowledge that, although this pragmatic approach is a widely accepted one, it does not consider important fundamental synecological processes at this point.	
4	Overall, this document clearly outlines the methods, data, and analysis by EPA used to derive the WQC therein. The assessment is for the most part comprehensive with respect to the available data and use of these data in a manner consistent with our scientific understanding of the ecotoxicology of Se in aquatic systems. I found the specific recommendations regarding WQC to be scientifically sound.	EPA thanks you for your review and comment.
5	The authors of this draft Technical Support Document (TSD) are to be commended for the breadth and detail of their effort. Also, with rare exception, the clarity of the presentation is outstanding. I found no fault with Parts 1 and 2 except for the conceptual model (2.7.3) being incomplete	EPA acknowledges that toxicity studies have been conducted on a limited number of aquatic-dependent bird species. However, through review of all available

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	(detailed below). Part 3 closely follows EPA's 2016 final criteria document for aquatic life and is therefore straight-forward and clear. Parts 4 and 5 present the core basis for the aquatic-dependent wildlife analyses. Except for several of the diet composition profiles (detailed below), the data presented are accurate. Perhaps the most critical untested assumption of the analyses presented in Part 4 is that the sensitivity of mallards to selenium will be sufficiently protective of roughly 95% of aquatic-dependent species of birds (and other aquatic-dependent taxa of wildlife). In reality, toxic sensitivity to selenium has been examined for very few species of aquatic-dependent birds. Thus, although mallards are on the sensitive end of those few, we still have very little insight as to the global species-sensitivity position of mallards. This should be a concern that the draft TSD explicitly addresses via an uncertainty (safety) factor or other appropriate means. For decades mallards were similarly viewed as a "sensitive" species for mercury toxicity. In the case of mercury, that assumption was recently tested (Heinz et al. 2009) and when a much broader range of bird species were tested for relative sensitivity mallards were revealed to be a fairly tolerant species (more tolerant than 50% of the newly tested species). The previous untested assumption about Mallard sensitivity to mercury was badly in error. The protocol used by Heinz et al. (2009) could easily be applied to testing EPA's assumption about the relative sensitivity of mallards to selenium, and until that kind of validation study is conducted, the high uncertainty regarding the level of protectiveness provided by a mallard model needs to be explicitly accounted for. Another major concern I have is the pooling of toxicity test results from studies with vastly different performance of controls; that simply should not be done (more below). I am also concerned about the implications of using mean or median values for key components of the of the criteria derivation process such as EF's, TTF's and diet composition. Means (approximately) and medians (exactly) correspond to only 50% protective values. Most	toxicity literature, including both controlled experiments and field studies, EPA determined that mallards were the most sensitive species to selenium exposure for which toxicity data exist. Additionally, through this literature review EPA found that waterfowl are the most sensitive taxa to selenium exposure (Janz et al. 2010; Ohlendorf 2003), based on available data. Therefore, as mallards were found to be the most sensitive species and waterfowl is believed to be the most sensitive taxa to selenium exposure, EPA believes that mallard is an appropriate surrogate to ensure the protection of closely related bird species inhabiting California. While recent avian mercury toxicity studies suggest that mallards are not the most sensitive species tested species to mercury, as was previously thought, similar data does not currently exist for selenium. Also, due to the differences in the mode of actions between selenium and mercury toxicity in birds, EPA did not use the recent findings by Heinz et al. (2009) for mercury to adjust the relative sensitivity of birds exposed to selenium. Therefore, as current toxicity literature indicates that waterfowl (and in particular mallards) are the most sensitive

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	observers would view 50% protection as an insufficient level of protection (including EPA, as illustrated by their choices of 90% protection (EC-10's) for toxicity testing data and 95% protection (SSD 5th percentile species)). Accordingly, components of the criteria derivation process should be based on more protective values for EFs, TTFs and diet composition. This would greatly improve the soundness of conclusions presented in the draft TSD. While I would agree that it is scientifically sound to assume that threatened and endangered species (T&E species) are no more nor less sensitive to selenium than species not so designated, the level of protection legally required for T&E species under the Endangered Species Act is a no effect (zero harm) standard. EPA's use of EC-10's for fish and avian toxicity and the 20th percentile of water values linked to fish and avian EC-10's are inherently not sufficiently protective for T&E species. All of the issues I've raised that bear on the soundness of conclusions can be corrected.	taxa to selenium exposure and as this literature does not indicate any differences in relative sensitivity between bird species (as is the case with other chemicals like mercury), EPA concluded that the bird criterion derived from mallard toxicity data would be protective of aquatic-dependent wildlife inhabiting California and no additional steps were taken to adjust the bird element (such as the application of an uncertainty factor as the reviewer suggested, which itself can introduce additional uncertainty if there is no scientific basis for the selected value). Regarding the reviewer's comment on EPA's estimates of EFs and TTFs, EPA intended to create an unbiased distribution of site criteria values by using best estimates of EFs and TTFs. From that distribution, which is intended to represent the real world, EPA selected the lower 20th percentile value for the water criterion values to enable the comparison of these values to those translated in the 2016 selenium aquatic life criterion. The appropriateness of this approach is discussed in more detail in response to Question 5a, Reviewer 5.

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		In obtaining each site EF, EPA used the central tendency of the distribution of time-variable water concentrations occurring at the site. But in implementation of the derived criterion at each site, the upper tail of that time distribution is compared to the criterion concentration. That is, the 30-day 1-in-3-year ambient water concentration is compared to the criterion. Because of the time variability of water concentrations at each site, a site having an EF that just allows its central tendency water concentration to attain the criterion water element would not attain the water goal when applied to its upper tail (30-day 1-in-3-year) concentration. As a result, the site representing the 20th percentile of the distribution (i.e., the site having its central tendency value equal to the water criterion element) would not end up attaining the criterion because its upper-tail (30-day 1-in-3-year) concentration would exceed. Consequently, the water criterion element is more protective than the reviewer has indicated.

Charge Question 1 ***Please comment on the overall clarity of the document and construction as it relates to assessing the risk to aquatic-dependent wildlife in California***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
1	The document is easy to follow, well-written, well-cited and well-organized. The authors present a balanced review of a comprehensive collection of related subject area references. The appendices present detailed supporting information.	EPA thanks you for your review and comment.
2	The document was clearly written and was adequate in addressing risk to aquatic-dependent wildlife in California with the notable exception of coastal and estuarine fish species. Of particular concern are the impacts to anadromous and catadromous species where tissue-based criteria in saltwater environments were not well addressed.	The criterion is intended to be protective of freshwater systems, and not saltwater. Please see EPA's response to the comment under General Impressions, Reviewer No. 2 and Charge Question 3, Reviewer No. 2 for additional information.
3	In my opinion, this document is clearly written and methodically lays out the details underlying the assessment for aquatic-dependent wildlife. The heavy reliance on mallard data is understandable and well-justified. Emphasis on bird egg hatchability is also reasonable (but see 2 below). The only unclear issue is that associated with uncertainties in estimates and their propagation relative to predictions. Measures of prediction uncertainties are inconsistently discussed. For example (page 42, 1st paragraph), an egg EC_{10} value is predicted from a logistic modeling of four mallard studies with no indication of the associated estimate uncertainty. It is then compared to other predicted values that also have no associated uncertainties given. It is hard to tell if there are actually differences among predictions or they all have overlapping uncertainty for estimates. The figures depicting some predictions from data such as Figure 4-1 do show confidence intervals. Such confidence intervals are very helpful to a reader and more consistent presentation of uncertainties would be	Confidence intervals are now reported for the updated egg EC_{10}. Regarding the other figures in Part 4.4 of the TSD depicting EC_{10} calculations; these figures were taken from other data sources, and confidence intervals are presented only if they were calculated and reported in the original source document. Figures where confidence intervals were not provided by the original authors are now noted in the respective figure captions. In addition, the text on page 48 regarding the interpretation of confidence intervals has been edited. NOEC/LOEC values are only presented in Part 4.6. The introductory paragraph to this section states: "To provide additional

Charge Question 1 ***Please comment on the overall clarity of the document and construction as it relates to assessing the risk to aquatic-dependent wildlife in California***		
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	helpful. As a related minor issue, discussion of uncertainty of mallard hatch vs. egg concentration (Page 48, paragraph 1) includes the statement, "*By contrast, all treatment concentrations greater than the 12.5 mg Se/kg dw egg EC_{10} would be within the variability of background (control) responses [based on control confidence limits], and that selenium concentrations up to 12.5 mg Se/kg dw would not lead to additional reductions in hatchability beyond natural conditions*." The wording might be changed because it seems to rely on a common misinterpretation of what a confidence interval is. Strictly speaking, if the process were repeated many times, 95 out of 100 of the resulting calculated intervals would contain the true control mean hatchability. It does not indicate that there is a 95% chance that the true mean is contained within a calculated interval. A credible or highest density interval would be more appropriate for making the inferences attempted here. Considerations of Pelecaniiformes, Strigiformes, and Passeriformes, and non-reproductive studies of Anseriformes summarize published effects studies and provide associated NOEC/LOEC metrics. Given the published criticisms of NOEC/LOEC metrics and steady movement away from their regulatory use, their use could prompt a distracting debate of their shortcomings. This could be avoided by omitting, or qualifying, their discussion.	evidence of the observed toxicity and effects of selenium, including the relative sensitivity of the bird species studied compared to mallards, these studies are presented below, divided into those with reproductive effects and non-reproductive effects and grouped by order." EPA recognizes the shortcomings of NOEC/LOEC values and has added the following sentences to the introductory paragraph in Part 4.6. "NOEC and LOEC values are provided in several of the following studies as a representative effect concentration for a comparison to the EC_{10} value calculated for mallards. The NOEC/LOEC values were not used in any quantitative analysis toward the determination of the final chronic value for aquatic dependent birds."
4	The overall clarity of the document is quite good. It follows the general risk-based paradigm that EPA has used in the past ~5 years for other WQC including the national WQC for Se. Having said that, there are a few areas which I detail below where clarity could be improved.	EPA thanks you for your review and comment.

Charge Question 1 ***Please comment on the overall clarity of the document and construction as it relates to assessing the risk to aquatic-dependent wildlife in California***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
5	The overall clarity and construction of the document are excellent. The document is easy to follow and its construction, i.e., general approach, makes sense.	EPA thanks you for your review and comment.

Charge Question 2 ***Please comment on the technical approach used to derive the draft criteria for aquatic-dependent wildlife presented in EPA's Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California. Is the technical approach used to derive the aquatic-dependent wildlife criteria logical? Does the science support the conclusions? Is it consistent with the protection of aquatic-dependent wildlife?***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
1	In my reading and analysis of the Criteria Document, the technical approach is logical and defensible. The work takes the available knowledge base, such as avian reproduction studies, and leverages that with the recently finalized USEPA selenium criteria and the significantly peer-reviewed USGS Ecosystem-Scale Selenium Model in a straight-forward manner to develop criteria protective aquatic life and aquatic-dependent wildlife including threatened and endangered species.	EPA thanks you for your review and comment.
2	The technical approach of using tissue based data to set site specific criteria for selenium is necessary. However, a better discussion of the uncertainties associated with this approach is needed. To use a single criteria value for all fish regardless of life history or spawning behavior does have uncertainties. Overall though, the procedure used to establish the value appeared to be sound for freshwater species. In addition, back calculating tissue-based criteria to water column values with a probabilistic method was a logical approach to derive site-specific values for selenium in freshwater systems. However, extrapolation or estimates in estuarine systems is poorly addressed primarily for aquatic organisms that may be influenced by	The development of a site-specific water column value can be achieved using methods described in the Performance Based Approach (PBA) discussed in *Translation of Selenium Tissue Criterion Elements to Site-Specific Water Column Criterion Elements for California* and in Appendix K of the 2016 ALC (U.S. EPA 2016). This approach for developing site-specific water column values involves consideration of the specifics of selenium

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	salinity. The uncertainty for these calculations is largely due to a lack of data in animals in saltwater systems compared to freshwater.	speciation, water body characteristics (e.g., lotic or lentic) and bioaccumulation via a specific food web in an ecosystem. The primary approach for deriving site-specific values that address differences in species composition at a site, and hence potentially different values for tissue criterion elements, is the Recalculation Procedure (U.S. EPA 2013) which may require the addition of new data using species with unique life histories or spawning behavior as the reviewer commented. This procedure uses the same methods as described in the Aquatic Life Criteria Guidelines (Stephan et al. 1985), which outlines criteria derivation methodology, but recalculations are tailored to the species that occur at the site. This criterion document was not intended to address saltwater systems. Please see EPA's response to the comment under General Impressions, Reviewer No. 2 and Charge Question 3, Reviewer No. 2 for additional information.

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3	The general technical approach to criteria development involves a thorough and logical progression toward the best options. Given the present context for criteria development, the entire process is well-done and defensible. The criticism that follows should not be seen as detracting from the judgment just presented: it is intended to indicate a direction to be considered in future efforts when enough information is available to do so. This and other criterion documents aspire to protect ecological communities as reflected in the statement (page 99), "*The chronic selenium criterion is derived to be protective of the entire aquatic community…*" The conceptual model diagram (page 29) specifies "*population decline*" and "*loss of species & community structure/function change*" are the ultimate effects of concern. However, the evidence applied in this draft document is derived from individual organisms dosed with toxicant. Often such evidence of effects on survival, growth, reproduction, and development of individuals is produced in highly-structured laboratory tests. Such information can be poorly predictive of population or community consequences (e.g., Forbes *et al*., 2011, *Human and Ecological Risk Assessment*, 17, 287-299; Kammenga et al., 1996, *Functional Ecology*, 10, 106-111). Crucial ecological features are excluded from consideration such as those bulleted below. • Alterations to species interactions are not included. This might be of particular concern given the importance of community processes (i.e., trophic interactions) on selenium exposure. As one example, mallard feeding studies	EPA relies on the best available data to develop scientific assessments, such as aquatic life criteria. EPA develops aquatic life water quality criteria based primarily on data on effects of a chemical on survival, growth, and reproduction. There is currently not sufficient information on sensitivities across life stages and taxa to complete a scientifically robust population modeling effort for selenium. EPA encourages generation of additional data on the sensitivity of an additional bird taxa to support development of a comprehensive avian species sensitivity distribution (SSD). Data available suggest that egg-laying vertebrates appear to have the same selenium-sensitive life stage, thus somewhat reducing but not eliminating the value of population modeling. Inclusion of species interactions is not feasible given currently available information.

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	demonstrated that high selenium food is not consumed as readily as control or low selenium food. In the wild, lowered palatability might result in prey switching which, in turn, would modify the received dose of a species living in a community with different prey species options (see Figure 5-1). As a hypothetical example involving pertinent bays, perhaps early in the winter scoter and scaup that would normally feed in shallow waters rich in *Corbicula* (Suisun Bay and San Pablo Bay) might shift to other prey in the deeper parts of the Central Bay. • Adverse effects to reproduction for the most sensitive species (white sturgeon and mallard ducks) are not linked here to population effects as might be done using demographic or elasticity analysis. Such population analyses are possible as illustrated in these two publications: Beamesderfer et al. 2007. Use of life history information in a population model of Sacramento green sturgeon. *Environmental Biology of Fish* 79:315-337, and Heppell. 2007. Elasticity analysis of green sturgeon life history. *Environmental Biology of Fish* 79:357-368. This kind of analysis could yield important insights because the most sensitive stage of an individual's life cycle is not necessarily the most crucial one for determining population persistence (Forbes et al., 2010, *Ecological Applications*, 20, 1449-1455; Hopkin, 1993, *OIKOS*, 66, 137-140; Kammenga et al., 1996, *Functional Ecology*, 10, 106-111; Petersen and Petersen, 1988, *Ambio*,	With regard to the "rivet popper" hypothesis, any consideration of such concepts in the current assessment would be speculative. Past experience with water quality criteria suggests that EPA's derivation approach yields criteria that achieve their goals for protectiveness. See also the response to Reviewer 5 comments on this charge question.

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	17, 381-386; Walthall and Stark, 1997, *Ecotoxicology and Environmental Safety*, 37, 45-52). • The following statement is made on Page 22. *"In the context of the Clean Water Act, aquatic life and aquatic-dependent wildlife criteria for toxic pollutants are typically determined based on the results of toxicity tests with aquatic and aquatic-dependent organisms in which unacceptable effects on growth, reproduction, or survival occurred. This information is typically compiled into a sensitivity distribution based on genera, and representing the impact on taxa across the aquatic community. Criteria are intended to be protective of a majority of aquatic organisms in the community (i.e., approximately the 95th percentile of tested aquatic organisms or aquatic-dependent wildlife representing the aquatic community).*" When applying this method, it should be kept in mind that it does not consider some fundamental synecological processes. Forbes and Forbes (1993. *Functional Ecology*, 7, 249-254) and Hopkin (1993. *OIKOS*, 66, 137-140) highlighted this shortcoming several decades ago. Frampton et al. (2006, *Environmental Toxicology and Chemistry*, 23, 2480-2489) more recently found the approach insufficient for soil invertebrates. Based on the redundancy hypothesis, the approach assumes that a certain number of species can be lost from a community without any degradation of community functioning. An alternative hypothesis (rivet popper hypothesis) proposes that any loss of species weakens	

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	a community. The work of Naeem et al., 1994, *Nature*, 368, 734-737; Tilman, 1996, *Ecology*, 77, 350-363; Tilman and Downing, 1994, *Nature*, 367, 363-365; and Tilman et al, 1996, *Nature*, 379, 718-720, Tilman et al., 2006, *Nature*, 441, 629-632) provide more support for the rivet popper hypothesis than the redundancy hypothesis.	
4	The technical approach used by EPA is entirely consistent with the state-of-the-science for the assessment of Se impacts on aquatic-dependent wildlife. This technical approach has been used by numerous researchers to estimate site-specific risks and I think EPA has successfully adapted this approach for the derivation of WQC for the protection of aquatic life and aquatic-dependent wildlife.	EPA thanks you for your review and comment.
5	The technical approach used to derive the draft criteria is logical and follows the approach used in EPA's 2016 final selenium criteria document for aquatic life which was developed over many years and refined with the benefit of extensive peer review. I think the level of protection that the criteria will provide is unknown because the global relative sensitivity of mallards is so highly uncertain. This uncertainty alone means the proposed criteria lack a rigorous level of scientific validation that would support any conclusion regarding how consistent or inconsistent the proposed criteria would be with the protection of aquatic-dependent wildlife. Overall, I would judge the proposed criteria as insufficiently precautionary considering the level of uncertainty associated with the most important scientific assumption that the criteria are derived from, and combined with the results of the only known test of that assumption for another pollutant that is also highly bioaccumulative and asserts its effects	Selenium toxicity data were available for eleven bird species, representing nine families and six orders. EPA recognizes that selenium toxicity data for birds are limited in the number of species tested and that there are data gaps on both the family and order level. However, through extensive literature review of both controlled experiments and field studies, EPA concluded that waterfowl are the most sensitive taxa to selenium exposure and that mallards are the most sensitive species for which there are toxicity data and are thus appropriate to use in generating criteria. EPA thus considers

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	primarily via dietary exposure of hens and subsequent maternal transfer to eggs, i.e., mercury (Heinz et al. 2009).	mallard to be an appropriate surrogate avian species for selenium and the criterion derived from mallard toxicity data to be appropriately protective of aquatic-dependent bird species inhabiting California.

Charge Question 3 ***Please comment on the toxicity data for aquatic-dependent wildlife used to derive the aquatic-dependent wildlife criteria presented in the draft document. Were the data adequately used and sufficiently comprehensive to represent risks to aquatic-dependent wildlife? Were the data selected and/or excluded from the criteria derivation appropriately utilized? Are there relevant data that you are aware of that should be included? If so, please provide for consideration.***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
1	The criteria document uses the exhaustive and well developed, well defended (quality and study type suitable for inclusion) database developed in the 2016 fish-tissue based Final Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater coupled with a comprehensive avian toxicity database from USFWS studies and peer-reviewed publications in the scientific literature. There were no new toxicity studies found in my examination of publications in the Web of Science database for the January 2017 to the present date that had direct relevance to the scope of the Criteria Document.	EPA thanks you for your review and comment.
2	Generally, the toxicity data used to derive the criteria for fish and avian species were sound for freshwater systems. The avian species data were also sound given the sensitivity of Mallard ducks and the life history of the species that inhabits freshwater and estuarine systems. However, the toxicity data were lacking in estuarine species under estuarine water quality conditions. Data excluded from the document included several studies showing that euryhaline fish species have different responses after saltwater acclimation. For example, juvenile rainbow trout *Oncorhynchus mykiss* (representing Steelhead) acclimated to saltwater were less susceptible to dietary selenomethionine acute toxicity (Schlenk et al. 2003). The rationale for excluding this in the earlier USEPA criteria appeared to be that only a single oral concentration of selenium was used with 3 varied salinities. It is true that this study should not be used to compare with other freshwater studies. The point of that study was to vary	EPA recognizes that the studies cited by the peer reviewer do show differential sensitivity to selenium between freshwater and saltwater treatments to 30-day juvenile rainbow trout and Japanese medaka embryos (Schlenk et al. 2003; Kupsco and Schlenk 2016a and Kupsco and Schlenk 2016b). The exposure of selenium to these fish that showed effects was either a short term (7-day) dietary exposure of one very high selenomethionine concentration of 180 mg/kg to juvenile rainbow trout or high aqueous exposures (5 µM to 50 µM or about 1 mg/L to 10 mg/L, respectively) to medaka embryos.

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REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	salinity and look at the effect of hypersaline acclimation prior to selenium exposure. A second group of studies that were omitted used the euryhaline model fish species, Japanese medaka (*Oryzias latipes*). Embryos and larvae from fish that were acclimated to saltwater were significantly more susceptible to the developmental toxicity of selenomethione at very specific developmental stages (Kupsco and Schlenk 2016a). The enhanced toxicity under hypersaline conditions with these stages was consistent with the induction of enzymes (Flavin-containing Monooxygenases) that activate selenomethione to selenomethione S-oxide which undergoes reduction/oxidation cycling and depletes cellular defenses of glutathione (Lavado et al. 2012). The oxidative stress observed following glutathione depletion initiated endoplasmic reticulum stress and alters the expression of bone and cartilage genes specifically in the caudal peduncle of larvae where lordosis (S-tail) occurs following selenomethione exposure (Kupsco and Schlenk 2016b). Inclusion of these data particularly in the conceptual model within brackish water systems indicate enhanced developmental toxicity of selenium rather than diminished acute toxicity which is what is used for saltwater thresholds ~70 µg/L.	The studies used to derive the aquatic life tissue criterion element in the CA TSD or U.S. EPA (2016) were limited to maternal transfer studies. This decision was based on the findings of a group of 46 experts at the 2009 Pellston workshop in the area of ecological assessment of selenium in the aquatic environment that agreed the most important toxicological effects of selenium in fish arise following maternal transfer of selenium to eggs during vitellogenesis, resulting in selenium exposure when hatched larvae undergo yolk absorption (Chapman et al. 2009, 2010). The studies cited by the commenter do not meet the exposure requirements to be used in the criterion derivation. U.S. EPA (2016) does discuss anadromous fish (Pacific salmon) in Section 6.4.1. The unique exposure of anadromous fish are discussed and the relatively sensitive effect levels of growth on juveniles. The non-reproductive EC10 for *Oncorhynchus* growth (GMCV) was 9.052 mg/kg whole-body which is just higher than the whole-

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		body criterion element of 8.5 mg/kg dw indicating such species should be protected. Alternatively, as the commenter stated, site-specific studies can be conducted to determine if a euryhaline or migratory fish needs additional protection. Such studies would likely require exposure of selenium to the parents with effects observations on the offspring.
3	Yes. The data were used adequately and were appropriate for representing risks. Rules for data inclusion seemed reasonable. The use of p-values of 0.05 as discerning thresholds was conventional but, nonetheless, sufficiently arbitrary to require more justification.	We appreciate the reviewer's comment that the data inclusion rules used for TTF calculation were conventional and reasonable. These rules are that paired selenium concentrations in a consumer species and its diet increase linearly, and that the slope of the linear relationship is statistically significant. Some threshold for data inclusion was necessary, and it was decided that those rules should be simple, reasonable, and broadly applicable across a wide range of taxa, and across multiple trophic levels. These rules are consistent with those followed in the TTF (and conversion factor – CF) calculations

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REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
		performed in the 2016 selenium ALC (U.S. EPA 2016). Field data are less certain than physiological laboratory studies because paired selenium concentrations in consumer and diet taxa can only be inferred. Based on what is known about selenium accumulation in laboratory studies, we expect selenium concentrations in consumers and diets to increase (positive slope), and we expect the relationship between selenium in consumer and diet tissues to be linear across a gradient of selenium concentrations (significant slope). If those conditions are met, then the median of those consumer-diet ratios is selected to minimize effects of individual outliers.
4	EPA appropriately screened the available laboratory toxicity data for birds in developing the aquatic-dependent wildlife WQC. However, I was surprised that EPA did not evaluate any of the rather large field data sets available relating egg Se concentrations to hatchability or chick survival (Skorupa 1998, Adams et al. 2003). While these data sets are unlikely to change the recommended bird egg Se threshold of 13.1 mg/kg dw, they are important field validation data sets that should be given consideration in WQC derivation.	A discussion of the EC_{10} for stilt hatchability calculated from the Skorupa (1998) field data described in Adams et al. (2003) has been added to the TSD.

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REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
5	I am not aware of any selenium toxicity data for mallards that were not included in the draft TSD. Data selection and exclusion criteria are clearly articulated and justified. Because toxicity data for only one species of aquatic-dependent bird is being utilized to estimate a globally "protective" egg selenium criterion, the data are not sufficiently comprehensive to adequately represent risk to the broad category of aquatic-dependent wildlife. I also do not think the data were properly used to estimate an EC-10 for mallard egg hatchability. As much as the authors want to pool results from mallard toxicity studies spanning a wide range of control performance, this simply is improper; neither control adjusting treatment results to an unfitted estimate of control performance, or pooling data without control adjustment are valid approaches to estimating an EC-10 from pooled data across separate studies. The very reason for the practice of control-adjusting data in the first place, is because it is inappropriate to pool results from studies with differing control performance without first adjusting for those differences. However, there are statistically proper and improper ways of control-adjusting data (OECD 2006). To pool results from separate studies, a fitted control value for each of the separate studies should have first been calculated, and then those values should be used for control-adjusting the treatment results. This was not done. I also believe it was improper to ignore the evidence from Heinz et al. (1989) that the control treatment produced selenium-deficient eggs. The control eggs in that study averaged less than 1 μg/g dry weight. Such low mean egg selenium content is virtually unknown from selenium-normal natural environments (Skorupa and	Please see EPA response to reviewer number 5 comments above under Question 2 for response regarding toxicity data used in criterion derivation. Upon further analysis and review of the bird criterion element, EPA modified the approach used to estimate an EC_{10} from the mallard toxicity data. Based on this new analysis an EC_{10} of 11.2 mg/kg dw was derived. This new approach is still based on a pooled analysis of the data; however, it does not include control normalization. Instead, the new analysis utilized the dose-response curve package in R (Ritz et al. 2015) to estimate an EC_{10} and the control groups were treated the same as the treatment groups across the studies and control normalization was not done as is consistent with OECD (2006). Additional details on the new analysis of an EC_{10} of 11.2 mg/kg dw can be found in Part 4.3 of the TSD. Also, a Fisher's exact test was performed to determine if there were any statistically significant differences in egg hatchability across the different control groups in the four

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REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	Ohlendorf 1991). The fact that the next treatment level in that study produced a mean level of egg selenium typical of avian eggs in selenium-normal natural environments AND decidedly higher egg hatchability than the controls certainly suggests that the control egg hatchability was in fact depressed due to selenium deficiency. Thus, Beckon et al.'s (2008) biphasic model for estimating the EC-10 from the Heinz et al. (1989) data is mechanistically justified, and statistically it produced the strongest fit to the data of the alternative statistical models examined. The most scientifically defensible EC-10 estimate for the Heinz et al. (1989) study is Beckon et al.'s (2008) estimate of 7.3 µg/g dry weight. This is important because of the 4 mallard toxicity studies EPA relied on, Heinz et al. (1989) was the most rigorous, with the most treatment levels. Results of the other 3 studies are of questionable value to add to the Heinz et al. (1989) study because they had so few treatment levels that fitted values for a control response would have such tremendously wide confidence intervals as to provide a very dubious basis for control-adjusting the treatment results. The draft TSD attempts to discard the troubling results of Beckon et al.'s (2008) analysis by asserting that if the control treatment in Heinz et al. (1989) was selenium-deficient, then it must have been deficient for other nutrients as well… a 100% speculative assertion. The non-speculative facts are that the control eggs were indeed selenium-deficient compared to selenium-normal eggs in nature and that is the only nutrient we have any data for. The draft TSD further asserts that control egg hatchability among 6 available mallard studies "is high"; yet EPA had already excluded 2 of those 6 studies for having unacceptably	studies. From this analysis, only the control group from Stanley et al. 1994 was determined to be statistically different from the other three studies (Stanley et al. 1996; Heinz et al. 1987 and 1989). Therefore, as this reanalysis did not utilize control normalization, data from Stanley et al. 1994 were excluded from the distribution and the EC_{10} was estimated with data from three of the four mallard toxicity studies used previously. The data from these three studies exhibited similar control hatchability (range of mean hatchability across studies was 57.3 – 64.4%; Stanley et al. 1996; Heinz et al. 1987 and 1989). Additionally, EPA did not consider the biphasic model presented by Beckon et al. (2008) to be justified for the pooled mallard toxicity dataset or for Heinz et al. (1989) alone as it is difficult to determine that a hormetic effect exists given that the study designs of the mallard toxicity studies did not include selenium deficient diets. To determine if a biphasic relationship exists for selenium toxicity in

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REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	low control egg hatchability!! And although EPA found the control hatchability of 57.3% in the Heinz et al. (1989) study to be "acceptable", it barely exceeded their 52% minimum value required for inclusion of a study in their subsequent analyses. That could hardly be accurately described as "high" control egg hatchability, especially when other studies had control egg hatchability as high as 91.4% (see Table 4-1). Also, I find the argument derived from Figure 4-4 to be unpersuasive. The range of control performances from different studies has no direct relevance to the results of a particular study. The results of the Heinz et al. (1989) study are clearly best fitted statistically by a biphasic model regardless of what the control performances were in other studies. For these reasons, it is my opinion that Beckon et al.'s (2008) estimate of the Mallard EC-10 for egg hatchability (7.3 µg/g dry weight) is more scientifically defensible than the value of 13.1 µg/g dry weight proposed in the draft TSD. Thus, the water criteria would be overestimated by a factor of 1.79 (13.1/7.3). For example, the derived criterion of 1.8 µg/L would properly become 1.8/1.79 = 1.0 µg/L.	birds, a study design would need to consist of properly spaced out treatment groups that include deficiency, sufficiency, and toxicity. EPA did not consider the biphasic model justified for Heinz et al. 1989 for two additional reasons (1) the measured egg selenium concentrations were below background concentrations in natural environments (< 3.0 mg/kg dw) for both the control group (mean of 0.6 mg/kg dw) and the first treatment group (mean of 2.77 mg/kg dw) and (2) the second treatment group (mean egg selenium concentrations of 5.33 mg/kg dw) had similar hatchability compared to the control and only the hatchability in the first treatment group was higher than the control group. For all these reasons EPA was unable to determine if the increased egg hatchability in the first treatment group (65.0%) compared to the control (57.3%) was due to a hormetic effort or from a spurious result.

Charge Question 3

Please comment on the toxicity data for aquatic-dependent wildlife used to derive the aquatic-dependent wildlife criteria presented in the draft document. Were the data adequately used and sufficiently comprehensive to represent risks to aquatic-dependent wildlife? Were the data selected and/or excluded from the criteria derivation appropriately utilized? Are there relevant data that you are aware of that should be included? If so, please provide for consideration.

REVIEWER NO.	REVIEWER COMMENT	EPA RESPONSE
		EPA, in its 2011 Rationale for the EPA's action on the revisions to Utah water quality standards, further discusses incompatibilities of the Beckon model with the Stanley et al. 1994 and 1996, and Heinz et al. 1987 data.

Charge Question 4

Please comment on the approach used to derive the EC_{10} described in this draft document of 13.1 mg/kg dw based on mallard toxicity data. Is the EC_{10} of 13.1 mg/kg dw protective of aquatic-dependent wildlife?

REVIEWER NO.	REVIEWER COMMENT	EPA RESPONSE
1	The authors have done a convincing analysis and reanalysis of the major original Mallard studies of Heinz and Ohlendorf, some of which were performed decades earlier, and modification of 2011 EPA reanalysis into a generalized linear model for a better statistical fit. In the context of the work presented in Part 4.4 of the Criterion Document, and the similarity to the earlier work of Ohlendorf, the EC10 of 13.1 mg/kg dw is protective of aquatic-dependent wildlife.	EPA thanks you for your review and comment. As noted above, EPA conducted a further refined analysis of the mallard data and developed a revised EC10 of 11.2 mg/kg dw.
2	The approach to derive the EC10 for selenium toxicity from eggs was sound for mallards. Mallards have been shown to have the best data set and the most sensitive bird species with available data. Since this value is less than the 15.1 mg/kg dw value for fish gonadal tissue, the criteria suggest that the mallard value will be protective of fish as well. While this may be true for freshwater systems, there is still significant uncertainty in estuarine systems.	EPA thanks you for your review and comment.

Charge Question 4 *Please comment on the approach used to derive the EC_{10} described in this draft document of 13.1 mg/kg dw based on mallard toxicity data. Is the EC_{10} of 13.1 mg/kg dw protective of aquatic-dependent wildlife?*		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	Very few of the species used to calculate CF had saltwater lifestages.	
3	In my opinion, the approach provides an acceptable estimate at this time of a protective concentration. Explaining exactly how "*control normalized data*" were produced would be useful because several approaches exist. Also, it might have been worthwhile exploring other threshold or change point models, e.g., Adams et al. (2003, *Environmental Toxicology and Chemistry*, 22, 2020-2029), Chen and Gupta (2012, *Parametric statistical change point analysis, 2nd Ed.*, Springer, NY), or those in the R package drc (Dose-response Curves).	The mallard EC_{10} was recalculated to be 11.2 mg/kg dw using the R package Dose Response Curve (drc) (Ritz et al. 2015). Data were not control normalized for this updated analysis. The EC_{10} of 11.2 mg/kg dw is presented in the updated TSD as the bird egg criterion element. Additional details regarding the derivation of the bird EC_{10} can be found in Part 4.3 of the TSD.
4	The approach used by EPA to derive the EC10 of 13.1 mg/kg dw based on mallard toxicity data is appropriate and the results consistent with previous analyses conducted for the same purpose (Adams et al. 2003, Ohlendorf 2003). The derived threshold will be protective of bird species that have been tested to date. However, it is difficult to conclude definitively that the threshold will be protective of all aquatic-dependent wildlife given the relatively small number of species tested. I think it is worth pointing out that the bird egg threshold developed by EPA is similar in concentration to the previously derived fish egg threshold. The mechanism of action for Se is likely to be similar for all egg-laying vertebrates, and this provides some re-assurance that the proposed threshold is protective given that the fish egg threshold is based on a relatively large number of taxa. While EPA provides a nice summary in the text of egg toxicity data for other species, I think it would be useful for EPA to develop a table that summarizes the toxicity endpoints for all bird species for which data are available (similar to Table 3 in a WQC criteria document for the protection of aquatic organism).	Data tables for all individual studies that were used quantitatively (i.e., the Heinz et al. 1987, 1989; and Stanley et al. 1994, 1996 mallard studies) or qualitatively have been added as appendices. Separate qualitative tables for reproductive and non-reproductive studies were constructed. These tables list NOECs and LOECs based on diet and/or egg, as applicable. A bird genus sensitivity distribution (GSD) cannot be created because there are a limited number of genera tested and there are data gaps across the family and order level. The mallard EC_{10} can be compared to the results of the data tables, however, to show that among tested

Charge Question 4 ***Please comment on the approach used to derive the EC_{10} described in this draft document of 13.1 mg/kg dw based on mallard toxicity data. Is the EC_{10} of 13.1 mg/kg dw protective of aquatic-dependent wildlife?***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	While not all data sets may be amenable to development of an EC10, most a suitable for deriving NOECs (often greater than values) and some LOECs. Development of a bird GSD would make for a more transparent assessment of the level of protection provided by the mallard EC10.	species, hatchability is a sensitive endpoint and mallards are a sensitive species.
5	See my response to question 3 above.	EPA thanks you for your review and comment.

Charge Question 5a. ***Please comment on the use of the USGS Ecosystem-Scale Selenium Model to derive the water column criterion elements for aquatic life and aquatic-dependent wildlife considering the fate and transport of selenium. In particular, please comment on:*** ***Any uncertainty surrounding the use of site-specific EFs (also commonly known as Kds) for California lentic and lotic water bodies***.		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
1	In my reading of the work, the approach for use and derivation of site-specific EFs for California lentic and lotic water bodies was done appropriately and consistent with best available knowledge. Although the knowledge base is not well developed in some areas (e.g., particulates) the authors appear to have been comprehensive in their approach.	EPA thanks you for your review and comment.
2	The USGS model was appropriate for deriving freshwater criteria. Separation of lotic and lentic systems was likewise appropriate using HUC categories. The use of 96 field study sites with particulate and corresponding water concentrations of selenium should have provided enough robustness to make estimates of EF. Again, the primary uncertainty here was that most were lotic/lentic and there did not appear to be any documentation of estuarine sites for comparison.	Please see EPA's response to Reviewer No. 2's comment under General Impressions. The document is now focused on freshwater systems since only freshwater data were used derive the tissue-based criteria and translate the tissue-based criteria to water elements. EPA has revised the document title to indicate applicability to freshwater only. For additional details regarding on how

Charge Question 5a. *Please comment on the use of the USGS Ecosystem-Scale Selenium Model to derive the water column criterion elements for aquatic life and aquatic-dependent wildlife considering the fate and transport of selenium. In particular, please comment on: Any uncertainty surrounding the use of site-specific EFs (also commonly known as Kds) for California lentic and lotic water bodies*.		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
		the proposed criteria are applied to waters in California, please see Section III of the proposed rule.
3	Estimation of enrichment factors appears sound and is clearly described. The use of medians was reasonable because a (heavily tailed) Cauchy distribution would be anticipated for the distribution of ratios.	EPA thanks you for your review and comment.
4	I think the approach used by EPA for EFs is reasonable.	EPA thanks you for your review and comment.
5	In my opinion, the use of the USGS Ecosystem-Scale Selenium Model is appropriate. However, to the extent that site-specific EF values were based on the mean or median of multiple EF measurements, the EFs would be only 50% protective. That is too low a level of protectiveness compared to EPA's normal choices for level of protection (see my response to question number 1).	EPA has characterized each site by best estimates of the applicable EFs and TTFs. The intent is to create an unbiased distribution of potential site-specific criterion values. From this distribution, EPA has selected a water criterion element concentration that is expected to protect a large percentage of sites, through the use of a 20th centile value selected from the distribution of water column values.

Charge Question 5b.
Any uncertainty surrounding the use of species specific TTFs in the food chain of aquatic life and aquatic dependent wildlife found in California.

REVIEWER NO.	REVIEWER COMMENT	EPA RESPONSE
1	The procedure outlined on page 79 using the approach and data described in Appendix B demonstrates a robust result consistent with the widely accepted USGS Ecosystem-Scale Selenium Model. *Page 79: EPA calculated avian TTFs following the general procedure described for the calculation TTFs in Section 5.3.1 above. Because five of the seven bird species consumed an omnivorous diet, the calculation procedure followed for fish was modified as follows. For species whose diet consisted of both plants and animals, information regarding species-specific dietary descriptions was used to calculate the relative proportions of the bird diet consisting of plants and animals. For every egg selenium measurement paired with additional selenium measurements from both aquatic invertebrates and aquatic algae and vascular plants, a weighted dietary selenium concentration was calculated. As with fish, paired data were required to be collected at the same site within a one year period (see Section 5.3.1 for additional details). Also following the approach used for fish, all paired invertebrate or primary producer species were included and considered as surrogates for dietary species from that trophic level. When more than one paired potential diet item from the same trophic level was available, the median selenium concentration was used.*	EPA thanks you for your review and comment.
2	The foodwebs were well characterized and the TTFs appeared to be appropriate for the model to estimate freshwater criteria. There were significant gaps with regard to estuarine systems, however. Nearly all species used in the TTF analyses were freshwater.	Please see EPA's response to Reviewer No. 2's comment under General Impressions. The document is now focused on freshwater systems since only freshwater data were used derive the tissue-based criteria and translate the tissue-based criteria to water elements.

Charge Question 5b.
Any uncertainty surrounding the use of species specific TTFs in the food chain of aquatic life and aquatic dependent wildlife found in California.

REVIEWER NO.	REVIEWER COMMENT	EPA RESPONSE
		EPA has revised the document title to indicate applicability to freshwater only. For additional details regarding on how the proposed criteria are applied to waters in California, please see Section III of the proposed rule.
3	TTF derivation is described on page 70, "*Briefly, the approach includes designating the median of the ratio of matched pairs of selenium measurements as the TTF, but only if ordinary least squares (OLS) linear regression of those data resulted in a significant (Pq0.05) fit and positive regression coefficient.*" It is debatable whether the p-value for regression lines should be used to decide if the relationship between paired egg and diet selenium data was adequate for producing a TTF. A single or very few points strongly impacted the calculated p-value for data depicted on pages 151, 154, 175, 178, 182, and 186. For data depicted on pages 159, 160, 162, and 164, the p-values were strongly influenced by there being essentially two clusters of points. Perhaps PRESS (predicted residual sum of squares) might have been a better tool for deciding model adequacy for making predictions?	EPA recognizes that several of these plots could potentially be a concern if TTFs were modeled from regression curves, for reasons the reviewer described. However, the objective of the regression-based data requirements of a statistically significant positive slope was to provide a simple and broadly applicable filter to paired field data to indicate whether the data were sufficient to provide a reasonable estimate of how much selenium increases in consumer tissue compared to diet. For example, in plots with multiple clusters of points at low and high concentrations, the regression-based filter notes that overall, selenium in eggs increases with selenium in diet. Despite the variability in selenium within a given cluster, when selenium in diet is low, it is also low in bird eggs, and when selenium in diet is high, it is also high in bird eggs.

Charge Question 5b. ***Any uncertainty surrounding the use of species specific TTFs in the food chain of aquatic life and aquatic dependent wildlife found in California.***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
		To address the variability in these field data, an outlier analysis of paired egg and diet data was performed for every bird species for which a TTF was calculated. Outliers were removed across three separate "passes" of each dataset following log-transformation. TTF figures and tables have been updated accordingly following the external peer review. Outliers have been retained in their respective Appendix tables but identified as being outliers. Finally, recognizing that field data are both limited and variable, the TTF is calculated using a median ratio, which measures the central tendency without being subject to the assumptions of regression analysis.
4	It is unclear to me why EPA did not develop TTFs for mallards given they are the species on which the WQC is based and there are data available from both field and laboratory studies for this purpose. I recommend EPA develop a TTF for mallards. I think it would also be worthwhile for EPA to evaluate TTFs based on lab studies in which birds were fed selenomethionine. It would be worthwhile to compare these to field-based TTFs where possible. If it can be demonstrated that they are comparable, there are several	EPA searched for studies with field data where selenium measurements in bird eggs were paired with selenium measurements in potential diet species. Data were primarily from a set of seven USGS reconnaissance studies. Among these species were mallards, which were assessed to determine if a field-based TTF could be calculated. According to the

Charge Question 5b. *Any uncertainty surrounding the use of species specific TTFs in the food chain of aquatic life and aquatic dependent wildlife found in California.*		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	species that lack field data, but for which lab-based TTFs could be derived.	quantitative dietary information available from the Birds of North America web site (https://birdsna.org), mallard diets consists of approximately 90% plants and 10% invertebrates. Based on the available paired field data, after calculating an overall diet based on a 90% plant and 10% invertebrate diet, a mallard TTF meeting the data quality requirements described in Part (5.3.1) could be calculated, but a mallard egg to plant TTF could not be calculated. This was because the overall relationship between paired plant and egg selenium concentrations was poor, and plants comprised the majority of mallard diets. EPA does not consider TTFs calculated from laboratory studies where diets were spiked with seleno-DL-methionine to be appropriate for the translation procedure, as these are not realistic exposures that could be expected to occur in field conditions. These kinds of studies are appropriate for toxicity testing, but not for calculating TTFs. However, if a mallard TTF were calculated from the six mallard

Charge Question 5b. ***Any uncertainty surrounding the use of species specific TTFs in the food chain of aquatic life and aquatic dependent wildlife found in California.***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
		hatchability studies conducted at the Patuxent Wildlife Research Center (Heinz et al. 1987, 1989; Heinz and Hoffman 1996, 1998; Stanley et al. 1994, 1996), it would be 2.69. This assumes a 10% moisture content in feed and does not include control points, because dietary concentrations were nominal, and based on selenium added to diet for a given treatment. Because the experimental feed is consistent of plants, the composite TTF is also 2.69, and would not drive the water column criterion elements.
5	The TTFs for American Coot and American Avocet are systematically biased high. The relevant diet composition is the composition of a hen's diet during ovulation. The TSD uses a general "species" diet composition based on data that include results for cocks as well as results for cocks and hens from outside the period of egg ovulation. It is known that female American Coots during ovulation greatly increase their relative consumption of animal matter. The value of 20% used in the draft TSD is too low by at least 3-fold. Hen avocets during ovulation essentially feed on 100% animal matter. In both cases, these shifts in diet are due to the very high protein requirements for producing eggs. To the extent that TTFs were based on site-specific mean or median values for invertebrate and plant selenium concentrations and mean or median values for avian egg selenium concentrations, the TTFs would be only 50% protective. That is too low a level of protectiveness	The dietary compositions for American coot and American avocet were based on quantitative information summarized by Brisbin et al. (2002), and Ackerman (2013), respectively, obtained from Birds of North America website (https://birdsna.org). Seasonal adjustments to the relative proportions of plant and animal food consumed were not made for these species because information was not available to support these differences for hens of this species. Regarding central tendency, EPA has characterized each site by best estimates of the applicable TTFs. The intent is to

Charge Question 5b. *Any uncertainty surrounding the use of species specific TTFs in the food chain of aquatic life and aquatic dependent wildlife found in California.*		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	compared to EPA's normal choices for level of protection (see my response to question number 1).	create an unbiased distribution of potential site-specific criterion values. From this distribution, EPA has selected a water criterion element concentration that is expected to protect a large percentage of sites, through the use of a 20th centile value selected from the distribution of water column values, and through application of the water criterion element to each site's highest 30-day, once-in-3-year concentration. It is not correct to characterize the result as only 50% protective.

Charge Question 6 ***Please comment on the science provided in EPA's Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California regarding the utility of the derived criteria for aquatic life and aquatic-dependent wildlife found in California with respect to the protection of listed threatened and endangered species from potential effects of selenium exposure.***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
1	The report "Species at Risk from Selenium Exposure in California Inland Surface Waters, Enclosed Bays and Estuaries" (U.S. FWS 2017) used as a background resource in the Criteria Document is a critical foundation for the application of the ecosystem Se model used in the derivation of the water quality criteria. The science appears as the best available knowledge in this area, and thus critical in the development of water column Se levels protective of threatened and endangered species.	EPA thanks you for your review and comment.
2	Overall, the science provided in this document was largely a duplication of the early 2016 freshwater document by EPA. It was unclear what additional science allowed extrapolation to saltwater systems. While the values submitted for avian species are likely protective, it is still unclear whether values in saltwater or anadromous or catadromous species of fish are protected by the values provided. **References** Kupsco, A. Schlenk D. (2016a) Stage susceptibility of Japanese medaka (Oryzias latipes) to selenomethionine and hypersaline developmental toxicity. Environmental Toxicology and Chemistry 35:1247-1256 Kupsco, A. Schlenk D. (2016b) Molecular Mechanisms of Selenium-Induced Spinal Deformities in Fish. Aquatic Toxicology 179:143-150.	Please see EPA's response to the comment under General Impressions, Reviewer No. 2 and Charge Question 3, Reviewer No. 2. The document is now focused on freshwater systems since only freshwater data were used derive the tissue-based criteria and translate the tissue-based criteria to water elements. EPA has revised the document title to indicate applicability to freshwater only. For additional details regarding on how the proposed criteria are applied to waters in California, please see Section III of the proposed rule.

Charge Question 6 ***Please comment on the science provided in EPA's Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California regarding the utility of the derived criteria for aquatic life and aquatic-dependent wildlife found in California with respect to the protection of listed threatened and endangered species from potential effects of selenium exposure.***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	Lavado, R. Shi, D. and D. Schlenk (2012) Effects of salinity on the toxicity and biotransformation of l-selenomethionine in Japanese medaka (Oryzias latipes) embryos: Mechanisms of oxidative stress. Aquatic Toxicology 108:18-22. Schlenk, D. Zubcov, N. Zubcov E. (2003) Effects of salinity on the uptake, biotransformation and toxicity of dietary seleno-l-methionine to rainbow trout. Toxicological Sciences 75:309-313.	
3	Based on the present available information, the criteria appear reasonable relative to protecting listed threatened and endangered species.	EPA thanks you for your review and comment.
4	The derived criteria should provide the same level of protection to T&E species as to other species. To the best of my knowledge, there is no evidence that T&E species are unusually sensitive to Se and they are likely to be randomly distributed within a general species sensitivity distribution for birds. The analysis by EPA for T&E birds is generally reasonable given the lack of data (other than dietary composition) for these species. While I appreciate the consideration of phylogenetic relatedness in assigning TTFs to T&E species, the data available for non-T&E species, though limited, provides no evidence that phylogeny drive TTFs. Indeed, both the highest and lowest TTFs are for grebes (Table 5-5). Given this, it might be more appropriate conservative to assume T&E species have a high TTF for this assessment rather than to assign TTFs based on phylogeny.	In accord with the findings of Sappington et al. (2001) (Environ. Toxicol. Chem. 20: 2869), EPA concurs with the reviewer's comment that "there is no evidence that T&E species are unusually sensitive to Se." Additionally, current literature suggests that there is no evidence that T&E species bioaccumulate selenium to a greater degree than other species. Consequently, in presenting its assessment of T&E species, EPA considers that it is important to present the best estimates of species vulnerability (sensitivity coupled with bioaccumulation propensity). Incorporating the reviewer's suggestion to apply a high-end TTF uniformly in the

Charge Question 6 ***Please comment on the science provided in EPA's Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California regarding the utility of the derived criteria for aquatic life and aquatic-dependent wildlife found in California with respect to the protection of listed threatened and endangered species from potential effects of selenium exposure.***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
		T&E species assessment would inappropriately imply a belief that T&E species had greater propensity to bioaccumulate selenium.
5	T&E species should be no more or less sensitive, on average, than non-listed species. Because ultimately we do not know what level of protection using a mallard model provides for other species of aquatic-dependent wildlife, the same would hold for T&E species. Even if a mallard model is sufficiently (95%) protective of other aquatic-dependent wildlife, the 13.1 µg/g dry weight EC-10 for mallards proposed here, in my opinion, would still be on the order of 1.8-fold too high for protecting T&E species. Even if mallard sensitivity were 95% protective and 13.1 µg/g dry weight were the best estimate of the mallard EC-10 for egg hatchability, water criteria based on an EC-10s and a 20th percentile value of EC-10 based site-specific modelling results would not meet the legal requirements of the Endangered Species Act which requires that proposed criteria be designed to meet a standard of no effect (zero harm) to all individuals of even the most selenium-sensitive T&E species. By design, the derived water criteria allow more than 10% harm to the most sensitive 5% of T&E species and to even a higher percent of T&E species at 20% of specific sites. In addition, an unknown percent of T&E species would be harmed at levels below 10%, but greater than 0%. For T&E species, EPA needs to estimate water criteria that would be "No Effect" concentrations. That is	Criteria are derived to protect designated uses as outlined in the CWA. With regard to T&E species, EPA notes that a 10% effect level is lower than the acceptable unexposed/control effect level for avian chronic toxicity experiments which allow up to 48% loss of hatchability in control/unexposed birds (U.S. EPA 2012). Thus, an increase of 10% effect is approaching the limit of scientifically-defensible effect calculations given the available data, and allowable hatching success in even unexposed birds in the experiments. Further, the 20th percentile value used for the water column criterion element analyses is the 20th centile of the distribution of the most bioaccumulative food chain for each site, and thus is inherently protective of 80% of even the most bioaccumulative systems. Thus, the combination of using the most sensitive

Charge Question 6 ***Please comment on the science provided in EPA's Draft Aquatic Life and Aquatic-Dependent Wildlife Selenium Water Quality Criteria for Inland Surface Waters, Enclosed Bays, and Estuaries of California regarding the utility of the derived criteria for aquatic life and aquatic-dependent wildlife found in California with respect to the protection of listed threatened and endangered species from potential effects of selenium exposure.***		
REVIEWER NO.	**REVIEWER COMMENT**	**EPA RESPONSE**
	sometimes estimated from the lower 95% confidence interval for the EC-10 (EPA 2000; Sparks 2000). Full citations for literature not already cited in the draft TSD: EPA. 2000. "Benchmark Dose Technical Guidance Document". [External Review Draft]. EPA/630/R-00/001. Environmental Protection Agency, Washington, DC. Heinz, G.H., D.J. Hoffman, J.D. Klimstra, K.R. Stebbins, S.L. Kondrad, and C.A. Erwin. 2009. Species differences in the sensitivity of avian embryos to methylmercury. Arch. Environ. Contam. Toxicol., 56:129-138. Sparks, T. (Ed.). 2000. *Statistics in Ecotoxicology*. John Wiley & Sons, Ltd, New York, NY.	bird species in the dataset and the most bioaccumulative food webs make this criterion analysis inherently protective under most conditions. EPA will also complete a formal ESA Section 7(a)2 consultation with NOAA Fisheries and FWS following completion of this document.

Specific Observations				
Reviewer No.	**Page**	**Paragraph**	**Comment or Question**	**EPA Response**
1			I have no specific annotated comments. However, my WORD program has a grammar checking product installed (Grammarly), and it notes numerous minor grammar errors that could improve readability.	EPA thanks you for your review and comment. The grammatical errors noted by the peer reviewers have been corrected in the revision to this document.
2	viii	last	How is embayment defined? SF Bay?	Please see EPA's response to the comment under General Impressions, Reviewer No. 2. Since only freshwater data were used to derive the tissue-based criteria and to translate the tissue-based criteria to water elements the criteria is applicable to freshwater only. EPA has revised the document title to indicate this applicability. For additional details on how the proposed criteria are applied to waters in California, please see Section III of the proposed rule.
2	18	first	ER stress Kupsco and Schlenk papers missing	EPA thanks you for your review and comment. The items listed have been added.
2	18	second	GSH peroxidases also detoxify lipid hydroperoxides	
2	20	First line 4	Depositied in liver then egg….vitellogenin is synthesized in the liver	
2	20	Line 9	Organoselenides are also metabolized by flavin monooxygenases	EPA thanks you for your review and comment. A statement was added to this section of the document recognizing the biotransformation of organic selenium by flavin monoxygenases (Palace et al. 2004).
2	20	Last line	For birds, diet and…	EPA thanks you for your review and comment. This item was corrected in the revision of this document.

Specific Observations				
Reviewer No.	**Page**	**Paragraph**	**Comment or Question**	**EPA Response**
2	23	Line 15	Selenomethionine exposure to eggs should be included	EPA is uncertain what the comment is addressing. It is not clear if the reviewer is referring to selenomethionine exposure through maternal transfer or eggs from reference fish that are subsequently exposed to selenomethionine. EPA made clear that dietary exposure is required in the tests it accepts. If the comment is referring to direct injection of selenomethionine into eggs, such tests are not acceptable for criteria derivation.
2	29		Nice conceptual model, missing saltwater influences	The document is for freshwater systems. Please see EPA's response to the comment under General Impressions, Reviewer No. 2 for additional information.
2	34	Last	Why not discuss uncertainties of taxonomic relatedness here?	EPA made the assumption that the distribution of selenium between tissues in fish (e.g., egg selenium relative to muscle or whole body selenium) is related to taxonomic similarity. For example, if a muscle selenium concentration is unknown for a salmonid species with a known egg selenium concentration, it is assumed using a salmonid distribution coefficient would provide a better muscle selenium estimate than an average coefficient for all fish. Because of limited data, EPA has not performed an uncertainty analysis.
2	53	Last first line	Selenomethionine spelling	EPA thanks you for your review and comment. The grammatical errors noted by the peer
2	57	Line 11	….endpoint were…	

Specific Observations				
Reviewer No.	Page	Paragraph	Comment or Question	EPA Response
2	57	Line 28	…all of the exposed…	reviewers have been corrected in the revision to this document.
2	58	Line 15	…nests was…	
2	58	Line 21	…were unaffected…	
2	58	Line 23	contaminants spelling	
2	74	first	Using taxonomy is uncertain. Carp and fathead minnow do not occupy the same ecological niche…they are the same family	EPA recognizes taxonomic relatedness has uncertainty but decided to use a consistent standardized procedure to calculate TTFs and conversion factors. Determining relatedness using the Integrated Taxonomic Information System (www.itis.gov) yields an unambiguous and reproducible result. In contrast, applying case-by-case best professional judgment about feeding guild would not yield a reproducible procedure. It may also be noted that because most selenium TTFs are not large, the end result is not particularly sensitive to how surrogates are chosen.
2	74	bottom	Uncertainties need to be discussed….assume asynchronous spawners? Same lipid content?? Same life stage?	Please see EPA's response to two of your comments above (Specific Observation comments from Reviewer No. 2, Page 34, last paragraph and Page 74, first paragraph). EPA discusses uncertainty of toxicity endpoints, conversion factors, TTFs, enrichment factors and water values in Section 6.3 of the 2016 ALC (U.S. EPA 2016) for selenium. This section does not address all the issues raised by the reviewer's comment, but it does address the variation among

Specific Observations				
Reviewer No.	**Page**	**Paragraph**	**Comment or Question**	**EPA Response**
				these parameters in a qualitative assessment of uncertainty.
2	102	first par	Nondetects were considered zero. Typically half of the detection limit or MDL is used rather than zero for non-detects	EPA thanks you for your review and comment. These data are ancillary information meant to be illustrative and has no influence on the criterion derivation.
2		2nd par	Typo "Error! Reference not found"? Two lines below….distributions were shown in..??	EPA thanks you for your review and comment. This paragraph has been deleted.
3	36-42		Numerous misspelled words and typographical errors.	EPA thanks you for your review and comment. The grammatical errors noted by the peer reviewers have been corrected in the revision to this document.
3	22	1	Uncertainty estimates for the SSD-derived value would be helpful.	EPA thanks you for your review and comment. Uncertainty estimates are not included in EPA criteria. Additional discussion of uncertainty can be found in Section 6.3 of the 2016 ALC (U.S. EPA 2016) for selenium.
3	23	2	Consider changing "traditional" to "conventional."	EPA thanks you for your review and comment. This change was incorporated in the revision to this document.
3	31	1	"The genus sensitivity…of invertebrate communities." Supply citation.	Part 3.2 of the TSD summarizes information from the 2016 aquatic life criterion document cited at the beginning of the section.
3	34	Table GMCV	Please provide uncertainty measures such as confidence intervals.	EPA thanks you for your review and comment. Confidence intervals are not included with acute and chronic species and genus mean criteria values.
3	53	2	Here and elsewhere, interpretations of NHST like this are coming increasingly under criticism.	EPA thanks you for your review and comment. Measures of selenium effects in these studies

Specific Observations				
Reviewer No.	Page	Paragraph	Comment or Question	EPA Response
				were NOECs, LOECs, and MATCs, and this section summarized those results.
3	56	2	Given the criticisms of NOEC/LOEC metric, consider omitting them or qualifying their use here and elsewhere.	The egg criterion is based on an EC_{10}. These studies are included as weight of evidence supporting the EC_{10} – based criterion. Studies where effects were reported using NOEC/LOEC metrics were treated qualitatively and are described as such in Part 4 and Appendix A of the TSD.
3	68	Figure 5-1	Consider removing figure.	EPA thanks you for your review and comment. This figure was changed to a bird-based food web figure in the revision to this document
3	74	2	It would be important to obtain this missing data for the next document revision.	EPA thanks you for your review and comment. Sufficient paired data for calculating species specific TTFs are not available.
3	87/93	1/1	Convention is mentioned as the reason for selecting a 20th percentile. A more science-based reason would be preferable.	The explanation provided in the revised document has been revised to be more explicit. The 20th percentile was previously used for the national criterion (U.S. EPA 2016). Note that the selection of the effects endpoint (i.e. specific percentile or level-of-protection) is a risk management decision.
3	99	2, 1st sentence	With the individual-based effect metrics, it is uncertain if the entire community will be protected.	EPA thanks you for your review and comment.
3	102	2	Please remove "Error: Reference source not found."	EPA thanks you for your review and comment. This error message resulted from a broken link and all broken links have been corrected in the recent revisions of the document.

Specific Observations				
Reviewer No.	**Page**	**Paragraph**	**Comment or Question**	**EPA Response**
4	3	2, Line 4	The 2016 criterion really has three tissue elements – egg/ovary, muscle, and whole body	EPA thanks you for your review and comment. The statement is correct as written.
4	6	1, Line 3	Algal transformations should also be mentioned here. See Simmons and Wallschlager (2011) and LeBlanc and Wallschlager (2016) for discussion.	EPA thanks you for your review and comment. Algal transformations and the two references have been added.
4	11	Fig. 2-3	This map would be more useful if it had bins of 0-1.5, 1.5-3, 3-10, >10 µg/L Se to be consistent with proposed and existing WQC.	EPA does not have the underlying data required to edit this map as requested. The map is ancillary information, used to help inform the reader and ultimately as no influence on the criterion derivation.
4	12	1, Line 3	True but re-phrase to acknowledge that Se is also depurated so a steady-state is reached unlike some POPs that cannot be depurated continue to increase in concentration throughout the life of the organism.	EPA thanks you for your review and comment. The steady-state phrase has been added to the recent revisions of the document.
4	12	1, Line 8	Unclear what is meant by "physical".	EPA thanks you for your review and comment. The word "physical," apparently intended to refer to dissolved and particulate, has been deleted as unnecessary as well as incompatible with the descriptor "wide range."
4	13	1, Line 11	Again, also see Wallschlager and colleagues references provided for page 6 comment.	Statements were modified, and Wallschlager and colleagues references added.
4	13	2, Line 1	The most important **aspect** of selenium….	EPA thanks you for your review and comment. This change was incorporated in the revision to this document.
4	13	2, Line 6	I don't think this is accurate. The primary organic selenide that has been evaluated is selenomethionine, which has much higher bioavailability than selenite (Kiffney and Knight 1990, Graham et al. 1992, Besser et al. 1993).	EPA thanks you for your review and comment. This was corrected to state that selenite and organic selenides are more bioavailable than selenate in algae.

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4	17	3, Line 2	Why "almost" all animals? Is there an example of an animal where Se is not essential?	EPA thanks you for your review and comment. A change was incorporated in the revision to this document and "almost" was deleted.
4	17	3, Line 3	Delete "the".	EPA thanks you for your review and comment. This change was incorporated in the revision to this document.
4	17-18	Last on p. 17 and on to p. 18	I appreciate that oxidative stress plays a role in Se toxicity, but the extent of its role is highly speculative at this point. We can measure oxidative stress, but mechanistically linking it to toxicity is rarely done and certainly hasn't be done for Se (i.e., more than measuring Se toxicity and oxidative stress at the same time which is correlative but not causative). For example, many other chemicals cause oxidative stress, why do they not cause the deformities we characteristically see in developing embryos? This paragraph should be more neutral and state that the mechanisms of Se toxicity are poorly understood and may be related to a number of processes such as….	EPA thanks you for your review and comment. A change was incorporated in the revision to this document including an added sentence stating another possible mechanism for toxicity/teratogenicity (disruption of endoplasmic reticulum homeostasis) and that more research is needed to understand selenium mode of action mechanisms.
4	17-18	Last, first sentence	These references are 12-20 years old. Not exactly "recent" as indicated.	EPA thanks you for your review and comment. This statement was changed to "more recent".
4	19	2, Line 17	I don't think there is quantitative evidence for 8, but if there is, why isn't this EPA's egg Se threshold. Given the large number of analyses that have been done on egg Se sensitivity to birds, with thresholds ranging from 8 to at least 16 ppm for mallards, more literature should be cited here, e.g., Adams et al. (2003). Also, it should be made clear	EPA thanks you for your review and comment. Edits were made as part of the revisions of this document to discuss deficiency and makes no statements about toxic thresholds for any species. Additional discussion of the EC_{10}s of Adams et al. (2003) has been added to Part 4 of the revised version of the TSD. Please also see EPA's

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			that these thresholds are for the most sensitivity aquatic-dependent bird tested to date, and not necessarily all bird species.	response to the comment under Specific Observations, Reviewer 4, page 44, Part 4.4.
4	20	Last, Line 4	Change to "For birds, diet and subsequently…."	EPA thanks you for your review and comment. The numerous grammatical errors noted by the peer reviewers have been corrected in the revision to this document.
4	21	2, Line 1	Delete "toxicity"	
4	22	3, Line 8-10	Delete "And" at beginning of sentence. Suggest re-phrasing to indicate mallards are the most sensitive aquatic-dependent wildlife tested to date.	
4	24	2, Line 15	Change to "These characteristically steep…	
4	24	2, Line 16	Delete "slightly". Stay quantitative.	
4	25	3, Line 4	Delete "system".	
4	27	Table 2.2	Change to "The chronic criterion…" in both instances.	
4	28	1, Line 3	This is the only reference in the whole document to neurotoxicity. I'm not familiar with the literature on this, but if it is a documented effect it should be discussed more thoroughly.	EPA thanks you for your review and comment. The mention of neurotoxicity was a mistake and has been removed in recent revisions of this document. Such effects were not observed in studies EPA used.
4	29	Figure 2.4	Is mining really the main source of Se in California as indicated by the weighted arrow. I would have though agricultural runoff was a bigger problem. If this is intended to be nationally rather than just California, it needs to be stated in the legend.	The conceptual model figure has been updated. The edits include the general reference to "point" and "nonpoint" sources instead of references to specific activities.
4	35	1, Line 3	Delete "of the bioaccumulation modeling approach".	EPA thanks you for your review and comment. The numerous grammatical errors noted by the
4	36	1, Line 7	Delete "in this chapter".	

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4	36	3, Line 2	"with mortality"	peer reviewers have been corrected in the revision to this document.
4	37	3, Line 1-2	"a reproductive"	
4	43	2	The modeling approach, i.e. model selection needs better description in this paragraph and/or in the paragraph at the top of page 49. It would be better to assess several different model types before selecting a specific model rather than saying simply that we used logistic regression because that is what was done in Ohlendorf (2003).	Model selection was an evolving process of revisions and refinements (e.g. adding additional parameters, control normalizing data, eliminating outliers) that was that result of consulting with technical experts in EPA and refined based on these external peer review comments. In the revised TSD, the selected model employed four parameters and did not require the data to be control normalized. The final model was assessed using significance tests on the individual parameters and goodness of fit tests on the model as a whole. These tests demonstrated acceptable p-values. Please see Part 4.3 of the revised TSD for additional details on the modeling approach used to derive the bird egg EC_{10}. The modeling approach described above and used to derive the bird egg EC_{10} in the revised TSD is conceptually similar to the approach used by Ohlendorf (2003), which is widely accepted for selenium and serves as the basis for the selenium standard in the Great Salt Lake of Utah (CH2M Hill 2008). Additionally, other previously published models were assessed before the modeling approach used to derive the bird egg EC10 was chosen. These previously published

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				modeling approaches are described in Part 4.4 of the revised TSD.
4	44	Figure 4.1	This figure should include confidence intervals for the regression and error bars for the data.	The 95% confidence interval around the regression curve has been added.
4	44	Part 4.4	This section should include a summary of the analysis performed by Adams et al. (2003).	Discussion of Adams et al. (2003) has been added to Part 4.4 of the revised TSD.
4	49	1, Line 3	Please provide more information on why this EC10 is a better statistical fit to the mallard data. What metrics were used and how did they compare to other analyses. As per my comment on page 43, the description of the statistical analysis and results needs to be more robust.	Please see EPA's response to the comment under Charge Question 4, Reviewer No. 3. EPA conducted a further refined analysis of the mallard data and developed a revised EC_{10} of 11.2 mg/kg dw. As part of this refined analysis of the mallard data EPA provided additional description of the statistical analysis in Part 4.3 of the revised TSD and comparisons to previously derived EC_{10}s in Part 4.4 of the revised TSD.
4	52	Part 4.6	This section should include the large field data sets presented in Skorupa (1998) and analyzed in Adams et al. (2003). A summary table of the effect levels from these studies should be provided.	A discussion of the stilt dataset presented in Skorupa (1998) and analyzed by Adams et al. (2003) has been added to Part 4.6 of the TSD.
4	55	2, Line 3	It is unclear what is meant by a "9% moisture diet". Do you mean a diet with a 9% moisture content?	EPA thanks you for your review and comment. This statement has been revised to state that there was a 9% moisture content in the diet.
4	64	2, Line 4	This bioaccumulation model was actually developed much earlier by Thomann (1981) and Connolly (1985). Please give proper credit.	EPA thanks you for your review and comment. The recommended citations have been added to the revised TSD.
4	65	1, Line 6-9	This last sentence if confusing with respect to the rationale for why TTFs do not need to be measured but EFs do need to be measured. Please clarify.	Additional sentences have been added to the revised TSD, citing Presser and Luoma (2010) explaining that TTFs are influenced by a species'

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				physiology and EFs are variable depending on local hydrology.
4	73	Table 5-3	Why were TTFs not developed for mallards and stilts? There are data from both the lab (mallards) and field (mallards and stilts) to do this. See, for example, data summarized in Adams et al. (1998) and Brix et al. (2005). EPA does not appear to have used laboratory data to develop TTFs. Is there a rationale for this?	A TTF for mallards was not developed from field data because the regression between available paired diet (modeled as 90% plants and 10% invertebrates) and egg data was not statistically significant. A TTF was not developed from laboratory data because selenomethionine is not a representative form of selenium found in the field. In addition, dietary concentrations among the mallard hatchability studies conducted at Patuxent Wildlife Research Center were not measured. Had a laboratory based TTF been calculated, excluding control data, where 0 mg Se/kg was added, it would have been 2.69. A TTF for stilts was not developed because paired field data for stilts that would allow for calculation of a TTF does not appear to be publicly available. However, a TTF of 1.44 was calculated for the closely related American avocet (Family Recurvirostridae) after incorporating additional paired data obtained since the external peer review and following an outlier analysis. TTFs for American avocet and all other available species are included in Appendix B.
4	80	Table 5-5	The highest and lowest TTFs are for two grebes suggesting variability within taxa is as great or	Presser and Luoma (2010) note that TTFs for fish all within a relatively narrow range (just over 3-

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			greater than across taxa and there is no phylogenetic signal. Consequently, for T&E species would it be better to simply use the highest TTF?	fold), particularly among taxonomically similar species. The relatively variable TTFs for eared grebe and pied billed grebe could be a consequence of limited data or could be a consequence of different diets. They differ by a factor of 2.56, within the range observed by Presser and Luoma (2010) across 25 fish species. In the TSD, the translated water criteria are based on the species with the highest composite TTF, in order to be protective to all bird species. In the TSD, this is the Ridgeways rail. Please see Parts 5.4.2 and 5.5.2 of the revised TSD for additional details.
4	80	2, Line 2	Wayland et al. (2006) provides data that allows for the calculation of TTFs for the American dipper.	Data from Wayland et al. (2006) were not used to calculate a TTF for American dipper primarily because egg and caddisfly data for reference sites were averaged across multiple sites, inconsistent with the practice of using paired data from a single location. Additionally, data from Wayland et al. (2006) were not used to calculate a TTF for American dipper because the reported dietary selenium concentrations were not consistent with the whole dietary composition of the species. Wayland et a. (2006) note that mayflies were an important component to the American dipper diet. Although mayflies were sampled and analyzed for selenium, results were not included. Finally, U.S. FWS (2017) indicates American

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				dippers also consume some fish; however, no fish were sampled in this study.
4	100	1, Line 4-6	This statement is overly definitive given how small the sample size is for fish-eating birds.	The statement has been revised. EPA thanks you for your review and comment
4	102	Part 6.3	I have concerns about the utility of this entire section. It is not clear to me that the data used in this analysis are based on a random spatially averaged sampling of State waters. Rather it appears there is a bias towards sampling waters with elevated Se. Is EPA really concluding that 67% of lentic waters in the State exceed the proposed WQC (Table 6.2)? If not and this is indeed a biased data set, the value of this analysis is unclear and inconsistent with typical WQC documents.	Please see EPA's response the comment under Specific Observations, Reviewer No. 2 (to page 74, bottom of peer reviewed TSD draft). Part 6.3 of the TSD that was peer reviewed was intended to be illustrative and did not influence the criterion derivation. This section has been revised to reflect peer review comments and moved to Appendix C in the revised TSD.
4	102	2, Line 4-5	There appears to be a reference problem.	EPA thanks you for your review and comment. This error message resulted from a broken link and all broken links have been corrected in the recent revisions of the document.
4	102	2, Line 7	Missing reference to Table at end of sentence.	
4	148	App. B	I did not go through in detail the studies that were and were not included in this assessment, but it appears that some of the study sites used in the analysis by Adams et al. (1998) (see their Table 1 for a list of sites used) were not included in Appendix B. This is consistent with the observation that EPA did not develop a TTF model for mallards despite data being available to do so as indicated in Adams et al.	All references listed in Table 1 of Adams et al. (1998) was examined, with the exception of Moore et al. (1989), which was not publicly available. Five studies from that list contained additional paired data that could be used to calculate TTFs. As a result, TTFs were updated for American coot and American avocet, and a new TTF could be calculated for Gadwall. Insufficient additional paired data were available to allow for the calculation of a TTF for mallards using a weighted plant+invertebrate diet. Next,

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				the list of references provided in the comment below was examined. The majority of these studies did not include the type of data required to calculate TTFs using previously established data requirements. Of these, Wayland et al. (2006) included paired data, but was ultimately not used because reference sites were averaged, mayfly selenium concentrations were not reported, and fish were not sampled. Please see EPA's response under Specific Observations, Reviewer No. 4 (page 80, paragraph 2 of peer reviewed TSD draft).
4			**Literature Cited** Adams, W. J., K. V. Brix, K. A. Cothern, L. M. Tear, R. D. Cardwell, A. Fairbrother and J. E. Toll (1998). Assessment of selenium food chain transfer and critical exposure factors for avian wildlife species: need for site-specific data. Environmental Toxicology and Risk Assessment: Seventh Volume, STP 1333. E. E. Little, A. J. Delonay and B. M. Greenberg. Philadelphia, Pennsylvania, American Society for Testing and Materials**:** pp. 312-342. Adams, W. J., K. V. Brix, M. Edwards, L. M. Tear, D. K. DeForest and A. Fairbrother (2003). Analysis of field and laboratory data to derive selenium toxicity thresholds for birds. Environ. Toxicol. Chem. **22**(9): 2020-2029. Besser, J. M., T. J. Canfield and T. W. LaPoint	As described in the previous response, the references cited here were examined. The majority of studies in this list were not used because they did not include the type of paired data required to calculate TTFs. Wayland et al. (2006) included paired data, but was not included for reasons described in the previous response.

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			(1993). Bioaccumulation of organic and inorganic selenium in a laboratory food chain. Environ. Toxicol. Chem. **12**: 57-72. Brix, K. V., J. E. Toll, L. M. Tear, D. K. DeForest and W. J. Adams (2005). Setting site-specific water quality standards using tissue residue criteria and bioaccumulation data. Part 2. Calculating site-specific selenium water quality standards for protecting fish and birds. Environ. Toxicol. Chem. **24**(1): 231-237. Connolly, J. P. (1985). Predicting single-species toxicity in natural water systems. Environ. Toxicol. Chem. **4**(4): 573-582. Graham, R. V., B. G. Blaylock, F. O. Hoffman and M. L. Frank (1992). Comparison of selenomethionine and selenite cycling in freshwater experimental ponds. Wat. Air Soil Poll. **62**: 25-42. Kiffney, P. and A. W. Knight (1990). The toxicity and bioaccumulation of selenate, selenite, and seleno-L-methionine in the cyanobacterium *Anabaena flos-aquae*. Arch. Environ. Contam. Toxicol. **19**: 488-494. LeBlanc, K. L. and D. Wallschlager (2016). Production and Release of Selenomethionine and Related Organic Selenium Species by Microorganisms in Natural and Industrial Waters. Environ. Sci. Technol. **50**(12): 6164-	

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			6171. Ohlendorf, H. M. (2003). Ecotoxicology of selenium. Handbook of Ecotoxicology. D. J. Hoffman, B. A. Rattner, G. A. Burton and J. Cairns. Boca Raton, Florida, Lewis Publishers**:** pp. 465-500. Simmons, D. B. D. and D. Wallschlager (2011). Release of reduced inorganic selenium species in waters by the green fresh water algae *Chlorella vulgaris*. Environ. Sci. Tech. **45**: 2165-2171. Skorupa, J. P. (1998). Risk assessment for the biota database of the National Irrigation Water Quality Program. Sacramento, California, U.S. Fish and Wildlife Service**:** 151 pp. Thomann, R. V. (1981). Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can. J. Fish. Aquat. Sci. **38**: 280-296. Wayland, M., J. Kneteman and R. Crosely (2006). The American dipper as a bioindicator of selenium contamination in a coal mine-affected stream in west-central Alberta, Canada. Environ. Monitor. Assess. **123**: 285-298.	
5	31	2	It seems scientifically indefensible to generalize to all amphibians from the results of toxicity testing for one species, the African Clawed Frog.	EPA thanks you for your review and comment. The sentence in question was edited in the revised TSD so that results of the African Clawed Frog toxicity test are not generalized to all amphibians.

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5	40	1	All of the mallard toxicity studies have low statistical power, thus, statistical significance is probably not as useful for risk assessment as "apparent effect" levels. For example, the Heinz et al. (1989) study reported a 10% reduction in egg hatchability at the 4 ppm dietary treatment level that produced mean egg Se of about 10 ppm. The relative reduction in egg hatchability would have been even larger than 10% for that treatment if a proper control were used, rather than the hatchability-depressed control for eggs with selenium-deficient selenium content.	Please see EPA's response to the comment under Charge Question 3, Reviewers No. 5. The mallard toxicity studies used to derive the bird egg EC10 were pooled into a meta-analysis to increase the statistical power of the analysis for mallard and the current toxicity data only support the derivation of a bird egg EC_{10} when the data from these multiple studies are pooled. Additionally, all toxicity studies used in the derivation of the bird egg EC_{10} met EPA's test guidelines and exhibited similar control hatchability. Therefore, EPA did not consider the control groups among these studies to be improper or the dietary treatment groups to be deficient.
5	29	Fig 2-4	Add arrows from producers to wildlife consumers 3rd trophic transfer and from consumers 4th trophic transfer to wildlife consumers 4th trophic transfer	EPA thanks you for your review and comment. These edits noted by the peer reviewer have been added to the figure in the revision to this document.
5	42	3	The practice of fudging control-adjusted hatchabilities of greater than 1 down to a value of 1 is far inferior to using a statistical model that accommodates hatchabilities greater than 1, such as the Beckon et al. (2008) biphasic model.	Please see EPA's response to the comment under Charge Question 3, Reviewers No. 5. The approach used to derive a bird egg EC_{10} has been modified in the revised TSD. This new approach is still based on a pooled analysis of the data. However, control normalization was not performed on the pooled data. Therefore, the downward adjustment of control hatchability values greater than 1 no longer applies. A single

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				EC_{10} was calculated from the three pooled studies using the dose-response curve package in R (Ritz et al. 2015). Pooled data included all treatment and control responses, and control normalization was not done, consistent with OECD (2006) recommendations.

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